

Histopathological Changes Induced by Monosodium Glutamate and Sodium Nitrite in the Development of Eye in Albino Mice *Mus musculus*

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Abstract

The present study aimed to discover the histopathological of the Monosodium glutamate (MSG), and Sodium nitrite (NaNO₂), on the embryonic development of the eyes of albino mice *Mus musculus*. On the fourteenth and eighteenth day of pregnancy, the stage of organogenesis in these animals. A concentration of 9 g/kg of MSG, a concentration of 110 mg/kg of NaNO₂, and the interaction between them used. The results of the study showed the presence of pathological changes to the eyes of the fetuses. The eye on the 14th day of pregnancy, when 9 g/kg of MSG used, there were retinal duplication, increased vascularization in the retina, condensation of some nuclei of the inner nuclear layer and ganglion cells, and necrosis in the vicinity of the lens. When treating with NaNO₂ 110 mg/kg, there was an irregularity in the lens, corneal distortion, hyperplasia of the retinal nerve tissue. When the two materials overlapped, the corneal tissue necrosis, the lens fiber, and the inner plexiform layer were observed. On the 18th day of pregnancy, when treated with MSG 9g/kg, the most significant overall and striking damage was retinal duplication and optic nerve necrosis. When treated with NaNO₂ 110 mg/kg, the corneal stroma and dissociation were seen in the photoreceptor cells. In the case of their overlapping, extensive necrosis and reduction appeared in all layers of the retina. The study concluded that consuming MSG and NaNO₂ more than the permissible limit during pregnancy leads to tissue lesions harmful to the eye.

Keyword: food additives, fetal development of the eye, MSG, NaNO₂.

التغيرات المرضية النسيجية المحدثة بكموتامات احادي الصوديوم و نترات الصوديوم في تطور العين للفئران المهق
Mus musculus

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الخلاصة:

تناولت الدراسة الحالية تأثير احدى المضافات الغذائية وهي كلوتامات احادي الصوديوم MSG، وحدى المواد الحافظة وهي نترتيت الصوديوم NaNO_2 على التطور الجنيني لعين الفئران المهق في اليوم الرابع عشر والثامن عشر من الحمل والتاثيرات المرضية النسجية التي تلحقها هذه المواد. بينت نتائج الدراسة من خلال الفحص المجهرى وجود تغيرات مرضية لعين الاجنة، فقد أظهرت العين في اليوم 14 للحمل وعند المعاملة بـ MSG 9غم/كغم ازدواجية الشبكية وهي من أهم الأضرار التي ظهرت في هذه المرحلة وهي نتيجة ملفتة للنظر ومهمة. أما عند المعاملة بـ NaNO_2 110 ملغم/كغم لوحظ عدم انتظام في العدسة، تشوه القرنية فرط تنسج في النسيج العصبي للشبكية وعند المعاملة بتداخل كلا المادتين لوحظ نخر في نسيج القرنية واليااف العدسة، وفي الطبقة الضفيرية الداخلية للشبكية. اما في اليوم 18 للحمل وعند المعاملة بـ MSG 9غم/كغم فقد كانت أهم الأضرار أيضاً الشاملة والملفتة للنظر أيضاً هي ازدواجية الشبكية فضلاً عن نخر العصب البصري. أما عند المعاملة بـ NaNO_2 110 ملغم/كغم شوهده تفكك في سداة القرنية وتفكك في الخلايا المستقبلية للضوء وفي حالة تداخلهما ظهر نخر واختزال واسع في كل طبقات الشبكية. واستنتجت الدراسة ان استهلاك هذه المواد اكثر من المسموح يؤدي الى حدوث افات نسجية في العين.

الكلمات المفتاحية: المضافات الغذائية، النمو الجنيني، كلوتامات احادي الصوديوم، نترتيت الصوديوم.

1. Introduction

Food additives can be defined as industrial or natural compounds added to food and are widely used in the food industries to increase the shelf life of the product and improve certain qualities in food, including coloring, preservation, and sweetening. Still, nevertheless, they have negative aspects, and some of them have been prohibited for their toxicity [1].

One of these substances widely used in the world is Monosodium glutamate (MSG), a substance that improves food flavor. It is a white crystalline powder [2]. It is the sodium salt of the non-essential amino acid Glutamic acid [3], symbolized as E621. It is used in the preparation of dried foods, canned soups, as well as in preserving frozen vegetables and fruits. It increases appetite and consumes large amounts of food, leading to obesity [4]. Also, glutamate and glutamate receptors contribute to an important role in many neurological diseases and pathological conditions of the eye, such as Ischemia and Trauma and Diabetic retinopathy, and Glaucoma [5].

The name of MSG is covered in foods under various names, including Umami or Ajinomoto. The US Food and Drug Administration considered it a safe and usable compound. The European Union classified it as a food additive that can be used in some foods, but according to specific conditions [6]. However, many studies recorded an increase in liver weight in rats given a diet containing MSG, which led to an increase in the activity of inflammatory tissue factors and led to hepatitis [7].

Sodium nitrite (NaNO_2) is also a food additive. It is a yellowish-white crystalline powder with unique pharmacological and industrial properties. It has been used in human and veterinary medicines as a vasodilator, antimicrobial agent, protection against hypoxia, and Preservative against bacterial contamination. [8]. NaNO_2 added to food as a preservative may interact in the stomach with amino compounds in food and form nitrosamine compounds that can cause cancer [9]. It may also contribute to the emergence of compounds and free radicals that accelerate fat oxidation, which causes dangerous

effects to various tissues and organs of the body. Therefore, NaNO₂ is a toxic chemical, and it is harmful when exposed to it, as it irritates the eyes, skin, and lungs [10].

The vertebrate eye is one of the most important and sensory organs in the body, so it has a complex structure and function, as it receives incoming light and turns it into images through which we can see the world around us [11]. The mammals' eyes are similar in structure to the rest of vertebrate eyes, as they consist of three main layers. The external is the sclera which contains the cornea. The middle layer is the Uvea, and the inner is the retina, which is the most important layer, and it is the eye photoreceptor layer because it contains photoreceptors. There are differences in vertebrates' eyes in terms of numbers and types of cells and their organization and arrangement [12].

The eyes are sensitive to teratogenic effects and infectious factors, as visual defects may result from an infection of tissues and organs with some microorganisms during the fetal period, such as rubella virus and syphilis, as well as the physical and chemical factors that a pregnant mother may be exposed to, which may lead to a defect in Eye formation and some defects that may affect the visual process after birth. Most ophthalmic defects result from damaged closure of retinal fissures such as coloboma of the iris, cataracts, and glaucoma may result from intrauterine infections [13].

As a result of the previous, and to investigate the effect of MSG and NaNO₂ on developing all parts of the eye in mice, this study was conducted.

2. Materials and Methods

- **Laboratory animals**

This study was conducted on Swiss white mice *Mus musculus*, and they were selected at an age ranging from (8-12) weeks, their average weight ranged (2 ± 23)g. They were placed in special laboratory conditions in terms of ventilation, temperature (2 ± 24) ° C and photoperiod (12 hours of light / 12 hours of darkness) [14]. The mice were fed continuously with their food [15].

- **Choosing of doses Concentration used in the study**

The dose concentrations were selected in light of the median lethal dose 50 (LD₅₀) for MSG ranging from 15-18 g/kg B.W. [16] and for NaNO₂ ranging from 85-150 mg/kg B.W. [17]. The doses that were used in this study were as following: (9 g / kg) MSG, the dose was administered in 0.5 ml D.W. orally by the Gavage needle, and (110 mg/kg) for NaNO₂ in 0.1 ml D.W. was given as intraperitoneal (I.P.) injection.

- **Experimental Design**

(24) pregnant mice were used, which were divided into four subgroups, as follows:

Duration of treatment	Groups	Material and concentration	Number of treatment days	Number of mice
From the seventh to the eighteenth day of pregnancy	Control	distilled water	12	6
	Group 1	MSG 9g /kg	12	6
	Group 2	NaNO ₂ 110 mg/kg	12	6
	Group 3	MSG 9g /kg+ NaNO ₂ 110 mg/kg	12	6

- **Histological sections preparation**

The histopathological sections were attended by anesthetizing and dissecting the pregnant mice on the 14th day and the 18th day of pregnancy for the control group and experimental groups mediated by

Chloroform. The embryos were extracted from the uterine horns [18] after gently removing the fetal membranes [19].

The eyes extracted from its orbit and the tissue sections were prepared by passing a series of steps, based on Suvarna *et al.* [20] as the following: Fixation was done by neutral formalin for 24-48 hours, after which it washed with water for 20 minutes. Then they went through an ascending series of ethyl alcohol at concentrations of 30, 50, 70, 90, and 100% for 15 min./ con. except for the last one for an hour for dehydration. They were clearing with xylene for 30 minutes. infiltration and embedding were done using paraffin wax, its melting point (54-56 ° C). Trimming and cutting were with a rotary microtome in a thickness of 5 µm. The histological sections were colored with Delafield's Haematoxylin and Eosin stain (H&E) based on Al-Hajj 's method [21]. The sections loaded with D.P.X. Finally, they were examination and imaging was done with a digital camera attached to a compound optical microscope.

3.Results

- **Histopathological changes in the eye of fetuses aged 14th days**

Microscopic examination of the histological sections of the fetuses' eyes at the age of 14th days showed the emergence of pathological changes compared to the control group (Figure 1,2). When pregnant were treated with MSG, increased vascularization was observed in the retina, condensation of some nuclei of the inner nuclear layer and ganglion cells, and necrosis in the vicinity of the lens (Figure 3). One of the most important damages that appeared at this stage is a duplication of the retina. Also, necrosis appeared in the area of the photoreceptor cells and the lens (Figure 4).

When pregnant mice were treated with NaNO₂, there was an irregularity of the lens, deformation of the cornea, thinning of the central part of it, hyperplasia of the retinal nerve tissue, and necrosis of the inner plexiform layer (Figure 5). This tissue damage may lead to a defect in the eye's fetal development process, which may ultimately cause a defect in the visual process after birth.

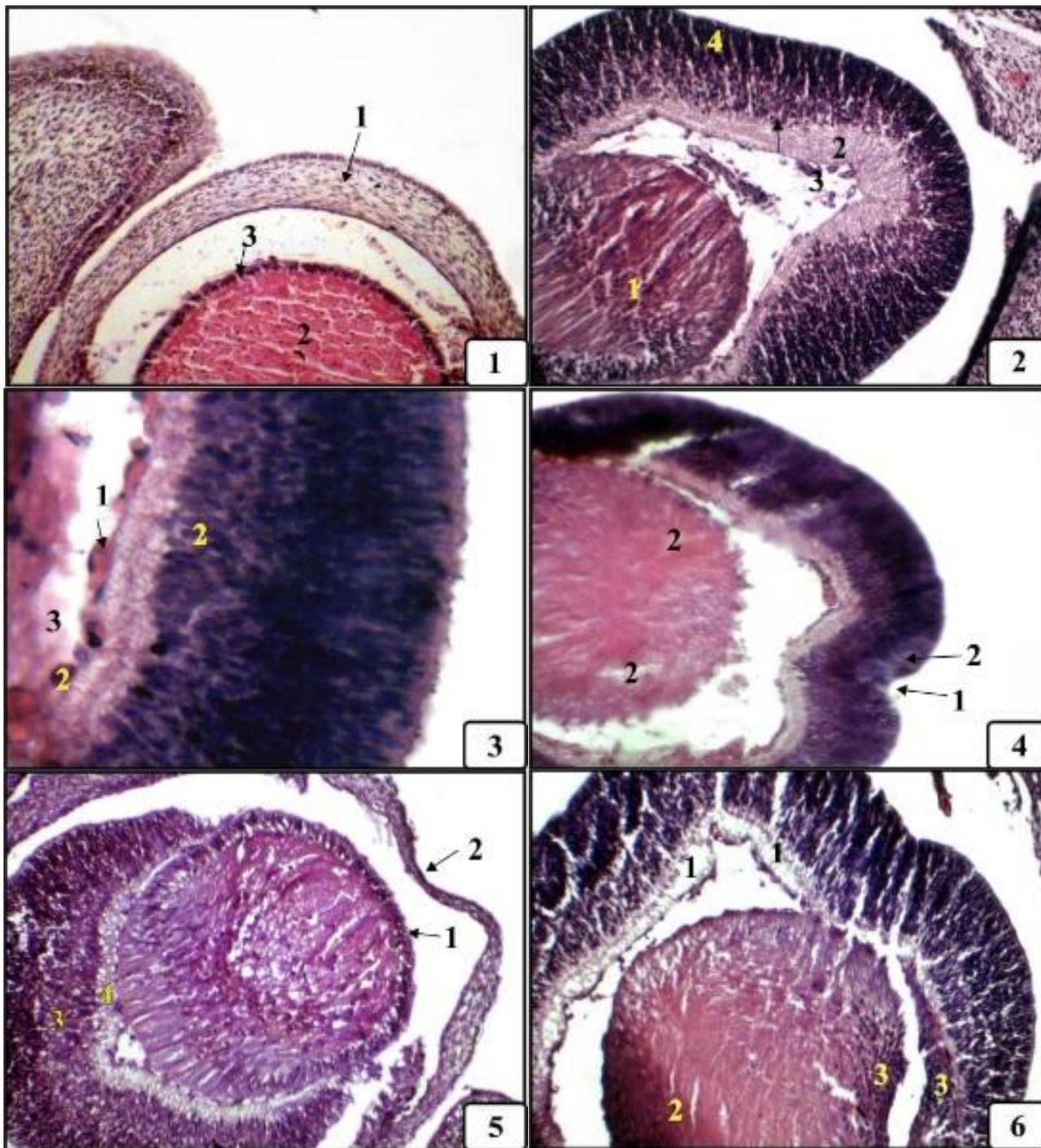


Figure (1): Histological section in the mouse's embryo eye (14th day of pregnancy) control group. 1: Cornea; 2: lens fibers; 3: lens capsule. 100X.; Figure (2): Histological section in the mouse's embryo eye (14th day of pregnancy) control group. 1: lens; 2: inner plexiform layer; 3: ganglion cells layer; 4: rest of retina layers.; 40X. Figure (3): Histological section in the mouse's embryo eye (14th day of pregnancy) group 1. 1: vascularization; 2: condensation of nuclei; 3: necrosis. 400X.; Figure (4): Histological section in the mouse's embryo eye (14th day of pregnancy) group 1. 1: retinal duplication; 2: necrosis. 100X.; Figure (5): Histological section in the mouse's embryo eye (14th day of pregnancy) group 2. 1: irregularity in the lens; 2: deformation of the cornea; 3: nerves tissue; 4: necrosis. 100X. Figure (6): Histological section in the mouse's embryo eye (14th day of pregnancy) group 3. 1: necrosis; 2: deformation of the lens; 3: infiltration of inflammatory cells. 100X.

In the case of the two substances being treated together, necrosis of the inner plexiform layer of the retina was observed, deformation of the lens with condensation of part of it, infiltration of inflammatory cells in a part of it, and the lining of the retina (Figure 6). also observed necrosis of the inner plexiform layer of the retina and increased awareness of the corneal tissue (Figure 7).

- **Histopathological changes in the eye of the fetuses aged of 18th days**

In the eye of 18th day old fetuses, several histopathological lesions were observed compared to the control group (Figure 8,9). When treated with MSG, the most striking damage was retinal duplication. Also appeared necrosis of the optic nerve and condensation of part of it (Figure 10) and in another part, in addition to retinal duplication, necrosis of the inner plexiform layer, reduction of the nervous tissue of a part of the retina, condensation of the lens (Figure 11). There was also a deformation of the lens, thickening of the epithelial tissue and its capsule, thickening of the cornea's inner lining, necrosis of the cornea's stroma, and deformation in another area of the cornea (Figure 12).

When treated with NaNO₂, the retina showed necrosis on the outer and inner nuclear layer, the inner plexiform layer, the dissolution of the photoreceptor cells, and the infiltration of inflammatory cells the edge of the inner plexiform layer (Figure 13). In another part of the eye, dissociation of the cornea's stroma fibers was observed, reducing the cornea's superficial epithelial layer, condensation of the periphery of the lens (the capsular), and hyperplasia of its epithelium (Figure 14).

When the treatment with both MSG and NaNO₂, extensive necrosis and reductions were observed in all retina layers (Figure 15). In the cornea, dissociation and necrosis of the stroma, reduction of the surface epithelium, and endothelial condensation were observed. Condensation of the lens's epithelium, hyperplasia, and necrosis of the fibers appeared (Figure 16).

4. Discussion

- **Histopathological changes in the eye of fetuses aged 14th days**

When pregnant were treated with MSG, many histopathological changes was observed, they included increased vascularization, condensation of some nuclei, necrosis in the vicinity of the lens, duplication of the retina, and necrosis in the photoreceptor cells and the lens. The duplication of the retina is one of the most important damages that appeared. It did not appear in previous studies and is considered a first-time result to the best of our knowledge. Some of these results are also consistent with the results that were performed on birds, as it was observed when treating chicken embryos during an incubation period with MSG causing them to have both morphological and retinal deformations, as the eyes were damaged, especially retinal tissues [22,23]

In a study on pregnant mice, they were treated with MSG, as they were dosed from day 17 of pregnancy at different concentrations 2, 4, 8 g / kg of body weight and studied the newborn's embryonic development mice`s cornea. The histopathological changes were observed during different periods, including the stroma fibers puncture, necrosis and reduction of the corneal epithelial layer, hyperplasia of the peripheral corneal epithelium, and part of it deformed, corrugation of the basement membrane of the central corneal epithelium, distortion of the fibers of the central corneal stroma, corrugation of the anterior surface of the cornea, reduction layering and irregularity, hyperplasia of fibroblasts, significant breakdown of stroma fibers, damage on the peripheral of the corneal epithelial tissue, hyperplasia of the central cornea, a reduction in the peripheral cornea of the generative cells for fibers, the separation of the endothelial epithelial tissue and the appearance of the condensed layer on the epithelial tissue [24].

When pregnant mice were treated with NaNO₂, there was an irregularity of the lens, deformation of the cornea, hyperplasia of the retinal nerve tissue, and necrosis of the inner plexiform layer. This tissue damage may lead to a defect in the eye's fetal development process, which may ultimately cause a defect in the visual process after birth.



In the case of the two substances being treated together, necrosis of the inner plexiform layer of the

Figure (7): Histological section in the mouse's embryo eye (14th day of pregnancy) group 3. 1: necrosis; 2: vascularization. 400X. ; Figure (8): Histological section in the mouse's embryo eye (18th day of pregnancy) control group. 1: cornea. 100X. Figure (9): Histological section in the mouse's embryo eye (18th day of pregnancy) control group. 1: retina; 2: lens. 100X.; Figure (10): Histological section in the mouse's embryo eye (18th day of pregnancy) group 1. 1: retinal duplication; 2: necrosis. 40X.; Figure (11): Histological section in the mouse's embryo eye (18th day of pregnancy) group 1. 1: retinal duplication; 2: necrosis; 3: reduction in neural tissue; 4: condensation in the lens. 40X. Figure (12): Histological section in the mouse's embryo eye (14th day of pregnancy) group1. 1: deformation of the lens; 2: condensation in the lens; 3: condensation in the cornea; 4: deformation. 100X.

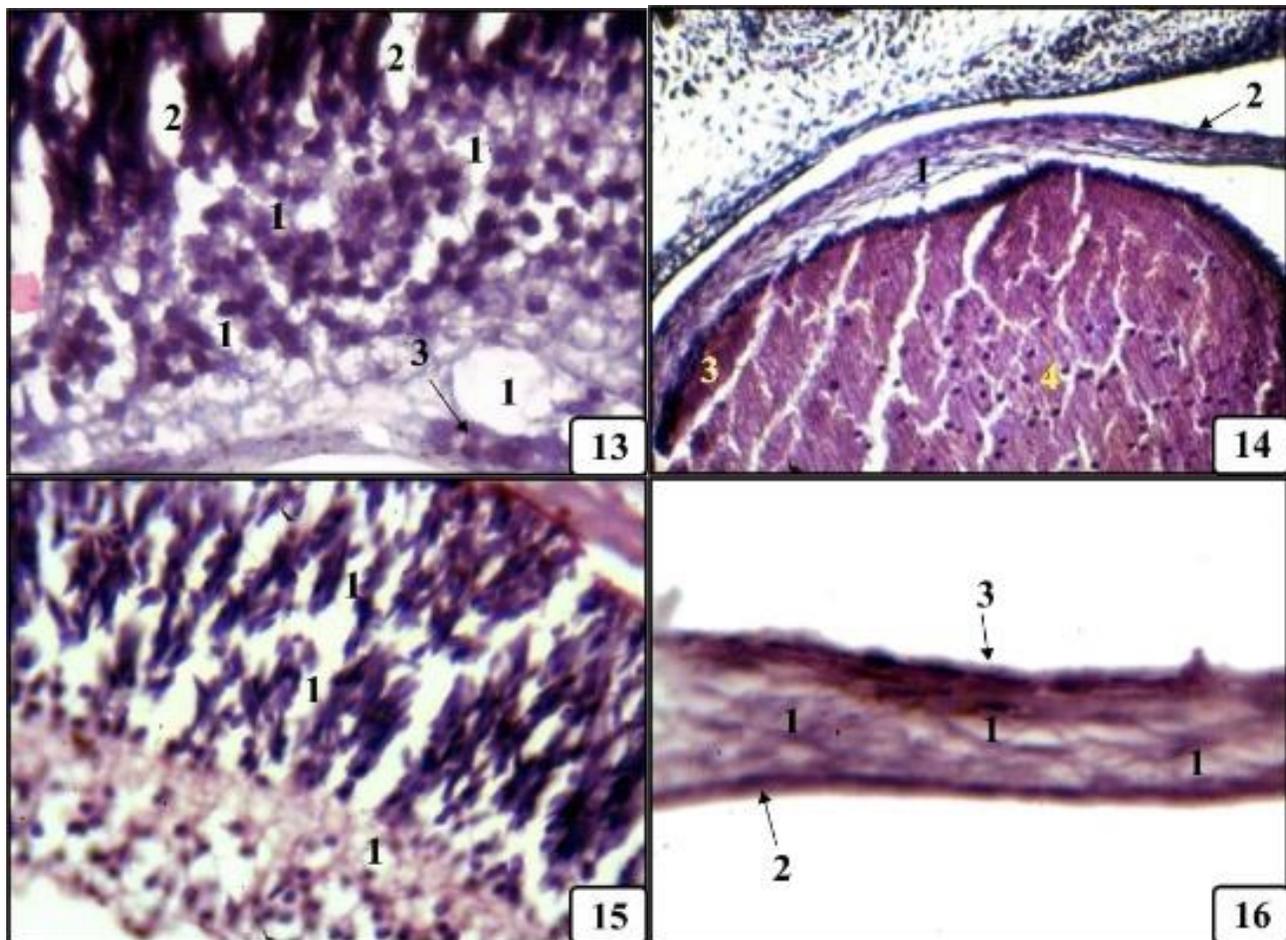


Figure (13): Histological section in the mouse's embryo eye (18th day of pregnancy) group 2. 1: necrosis; 2: dissociation in the photoreceptor cells; 3: infiltration of inflammatory cells. 400X. ; Figure (14): Histological section in the mouse's embryo eye (18th day of pregnancy) group 2. 1: dissociation in cornea fibers; 2: reduction in superficial epithelia; 3: condensation in the lens; 4: hyperplasia in lens epithelial. 100X. Figure (15): Histological section in the mouse's embryo eye (18th day of pregnancy) group 3. 1: necrosis; 2: large reduction. 400X.; Figure (16): Histological section in the mouse's embryo eye (18th day of pregnancy) group 3. 1: dissociation and necrosis; 2: reduction in superficial epithelia; 3: condensation in the endothelium. 100X.

retina, deformation of the lens, infiltration of inflammatory cells, and necrosis of the inner plexiform layer were observed. These results confirm what was stated in the study of [25], as they demonstrated the presence of damage to the tissues of the eye as a result of ingesting these substances, which led to the emergence of widespread damage to the eye and gave a recommendation for the need for treatment.

- **Histopathological changes in the eye of the fetuses aged of 18th days**

In the eye of 18th day old fetuses, several histopathological lesions were observed. When treated with MSG, the most striking damage was retinal duplication. Also appeared necrosis of the optic nerve, necrosis of the inner plexiform layer, reduction of the nervous tissue. There was also a deformation of the lens, thickening of the cornea's inner lining, and necrosis of the cornea's stroma.

When treated with NaNO₂, the retina showed necrosis, the dissolution of the photoreceptor cells, the infiltration of inflammatory cells the edge of the inner plexiform layer, dissociation of the cornea's stroma, reducing the cornea's superficial epithelial layer, condensation of the periphery of the lens, and hyperplasia of its epithelium.

When the treatment with both MSG and NaNO₂, extensive necrosis and reductions, dissociation and necrosis of the stroma, reduction of the surface epithelium, endothelial condensation, condensation of the lens's epithelium, hyperplasia, and necrosis of the fibers appeared.

This study's results are in agreement with what Ibrahim [19] indicated about the effect of MSG on eye development, as it showed the emergence of different histopathological lesions of the cornea, lens, and retina with its secondary layers. The results of the current study are also to the findings of Al-Najjar [26] regarding the effect of smoking on the embryogenesis of the eye on the eighteenth day of pregnancy, which was hyperplasia of the central retina, thickening of the sclera layer, lens distention, corneal thickening, necrosis of the inner plexiform layer and condensation of ganglion cells. We find other previous studies on the effect of MSG alone and NaNO₂ alone but not their overlap, and we believe that most of our results were recorded for the first time to our knowledge.

5. Conclusion

It can be concluded from this study that taking MSG and NaNO₂ above the permissible limit for pregnant women may lead to clear damage in most eye tissues during their fetal development and ultimately visual impairment, so we recommend avoiding their use, especially in concentrations close to the average lethal dose.

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